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On the Origin of the Frontal Gland of Amphibians*

With 2 Text-figures

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It has long been believed that the frontal glands of amphibians arise from the cells of the outer or the basal layer of the epidermis (Saguchi, 1915; Goda, 1929; Holtfreter, 1933; Lieberkind, 1937; Hama, 1946). Recently, Yanai (1950, 1951a, b, c) has carefully studied the normal development of the gland in various amphibians and come to the conclusion that the frontal glands are derived from the neural crest cells.

Holtfreter (1933) transplanted a piece of the presumptive epidermis of the early gastrulae of *Triton* and *Amblystoma* to the head of the neurulae on the outside of the frontal region, and observed that frontal glands were induced in the epidermis of the transplants. Hama (1946) grafted fresh head-organizer or heat-killed trunk-organizer to the early gastrulae of the newt, *Triturus pyrrhogaster*, and found that frontal glands were induced in the epidermis of the host's abdomen. From these observations, both investigators concluded that the frontal glands are of epidermal origin.

It should be remembered, however, that (1) in the early gastrulae, the neural plate has not yet been differentiated from the epidermis, and that (2) both neural plate and neural crest can be induced in the presumptive epidermis. The occurrence of frontal glands in the region of the epidermis which is normally lacking in these organs, therefore, does not necessarily show that the glands arise from the epidermis *per se*, since the possibility cannot be excluded that neural crest is induced in

* Studies on the Hatching Gland of Amphibians X.

the epidermis, and the neural crest cells in turn differentiate into frontal glands. Moreover, the experiments of both Holtfreter and Hama were done with urodele embryos. It is the purpose of the present study to determine experimentally the origin of the frontal glands in anurans.

Before going further, we wish to thank Professor T. Fujii of Tokyo University for valuable information, and also to acknowledge our indebtedness to Assistant Professor R. Tamura of this University for advice regarding the statistical treatment of experimental data.

MATERIALS AND METHODS

Neurulae of the toad, *Bufo vulgaris formosus*, and the frog, *Rana nigromaculata*, deprived of the jelly envelope in Holtfreter's solution, were subjected to further operations.

1. Experiment with toad neurulae (Fig. 1, A, B)

The neural crest was removed from neurulae immediately after the fusion of the neural folds. The technique was as follows. In the mid-dorsal region of the head, a ⊔-shaped flap of surface ectoderm (the outer side of the neural folds) covering the newly formed neural tube was cut (Fig. 1, A). On turning up the flap, the neural crest under the flap was divided into two layers and exposed, one on the underside of the ectodermal flap and the other covering the dorsal surface of the neural tube. Both of the layers thus exposed were carefully scraped off. The flap was then replaced.

At this stage of development, a black longitudinal line is seen on the dorsal surface of the embryo, along the line of fusion of the neural folds. The line seems to consist of neural crest cells which have thrust from below to the fusion line. This line, however, was left intact.

Unoperated neurulae and sham-operated neurulae, in which a flap of cephalic ectoderm was turned up, but was put back leaving the neural crest intact, both originating from the same batch of eggs as the experimentals, served as controls.

2. Experiment with frog neurulae (Fig. 2, A, B)

Of two groups of embryos from the same batch of eggs, one was allowed to develop normally in tapwater, and the other was kept in a 1/50,000 Nile blue solution.

Shortly before the fusion of the neural folds, a piece of tissue was taken out from the side wall of the neural groove of the stained embryos, after removal of the epidermis (Fig. 2, A). The piece was so prepared that it contained either neural plate material together with some neural crest cells (Fig. 2, B, a) or only neural plate tissue (Fig.

2, B, b). Care was taken to make the piece free from epidermal cells. The piece, after being placed in Holtfreter's solution for a while until it became compact, was grafted to the corresponding region of an unstained embryo.

Operated embryos of both the toad and the frog were reared in Holtfreter's solution together with unoperated embryos deprived of the jelly coat. Two days after operation, when the frontal glands had become maximally developed in the intact controls, embryos were fixed for histological study. Toad embryos were fixed in Bouin's solution, and sections were stained with Delafield's hematoxylin and eosin. With frog embryos, the Fyg and Baltzer method was applied. Embryos were fixed in acetic acid-free Zenker's solution, and dehydrated in corrosive sublimate-alcohol. Sections were stained with chrom-alum and acid alizarin-blue.

The method of cell-counting in sections has previously been described by Yanai (1951c). Data obtained by this technique are not absolutely accurate but are sufficient for the purpose of comparison. The confidence interval of the mean cell number was up to 95 per cent. The significance of the difference between the means was tested by the F-test at the 5 per cent level of significance.

RESULTS

1. Experiment with toad embryos (Tables 1, 2, Fig. 1, C, C′, D, D′, E)

In embryos from which the neural crest of the head had been removed as much as possible, the number of the frontal glands in the operated head region was distinctly smaller than in control embryos (about 62 per cent on an average). The difference between the means of experimental and control embryos was also statistically highly significant.

In experimental embryos N2, N4 and N5, in particular, the frontal glands of the head region were much reduced in number. Some of the serial cross sections of the head of these embryos were completely devoid of the frontal glands, while every cross section of the head of the control embryos contained numerous frontal glands, about 50 per cent of the total number of the glands being found in the head region.

Sham-operation failed to influence the number of frontal glands in the head region of the embryos when compared with intact controls, since the difference between the means appears statistically insignificant.

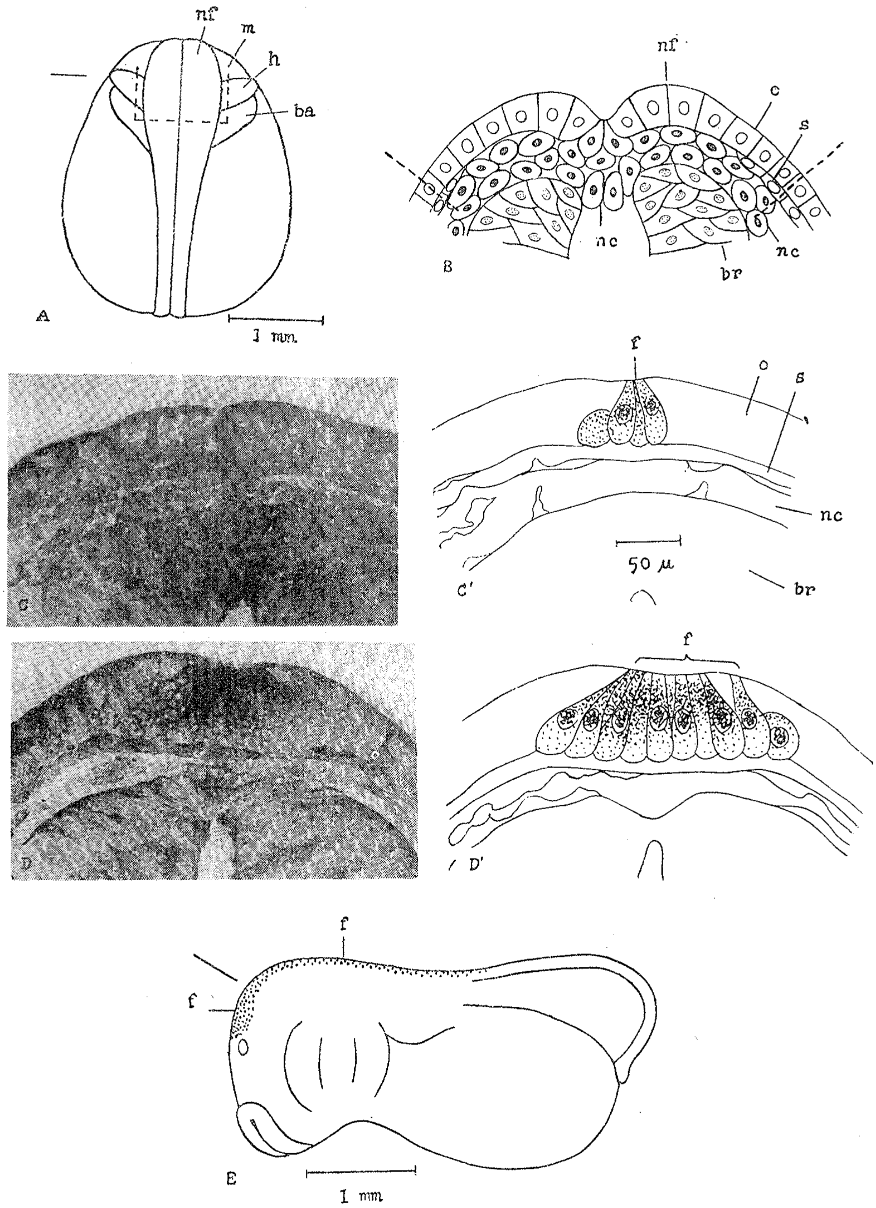

Fig. 1. Removal of neural crest from toad embryos. A, External appearance of neurula immediately after fusion of neural folds. B, Diagrammatic representation of cross section of anterior dorsal part of the same. Broken lines in Figs. A and B show planes of cutting at operation. C, C′ and D, D′, Sections of frontal region of embryos immediately before hatching. Direction of sectioning is shown by oblique line in upper left corner of Fig. E showing side view of normal embryo. C and C′, Embryo deprived of neural crest in earlier stage. D and D′, Intact control. ba, branchial arch; br, brain; e, outer layer of epidermis; f, frontal gland; h, hyoid arch; m, mandibular arch; nf, neural fold; nc, neural crest; s, basal layer of epidermis.

Table 1.

Reduction in number of frontal glands in head region* of toad embryos deprived of neural crest

Neural crest removed		Sham-operated		Control	
Case no.	No. of glands	Case no.	No. of glands	Case no.	No. of glands
N 1	182	F 1	289	C 1	169
N 2	92	F 2	276	C 2	300
N 3	190	F 3	222	C 3	255
N 4	93	F 4	244	C 4	246
N 5	136	F 5	282	C 5	258
N 6	193	F 6	267	C 6	273
N 7	229	F 7	215	C 7	300
N 8	170	F 8	221	C 8	254
		F 9	239	C 9	316
		F 10	184	C 10	286
		F 11	150	C 11	232
				C 12	346
				C 13	241
				C 14	264
				C 15	235
				C 16	294
				C 17	234
				C 18	241
				C 19	154
				C 20	270
Average	160.6 ±40.6		235.4 ±30.3		258.4 ±21.1

* The "head region" is the body portion contained in 50 serial sections, 10μ thick, beginning just behind the nasal pits and extending caudally. The anterior limit of distribution of the frontal glands coincides with the anterior limit of the head region. The posterior edge of the ectodermal flap is situated a little posteriorly to the caudal end of the head region.

Table 2.

Statistical comparison of mean numbers of frontal glands in head region of toad embryos deprived of neural crest and their controls

Operated		Control	F		Significance
			Observed	5% level	
Neural crest removed	160.6±40.6	258.4±21.2	25.17	4.22	Significant
Sham-operated	235.4±30.6		4.07	4.18	Insignificant

2. Experiment with frog embryos (Table 3, Fig. 2, C, D)

Nine of 11 embryos receiving a vitally stained transplant yielded available results. The remaining two became deformed during further development and were discarded.

In all of the nine embryos, cells derived from the Nile blue-stained transplants were readily distinguishable from unstained host cells. Transplanted neural plate formed either an integral part of the brain of the host or a second brain in close proximity to the host brain.

When some neural crest cells had been grafted together with the piece of the neural plate, some Nile blue-stained cells appeared not only in the brain but also in the frontal glands, the basal layer of the glands, and the mesenchyme of the host's head. On the other hand, in four of five embryos receiving a transplant composed of neural plate only, blue cells were encountered only in the brain and not in other places. One

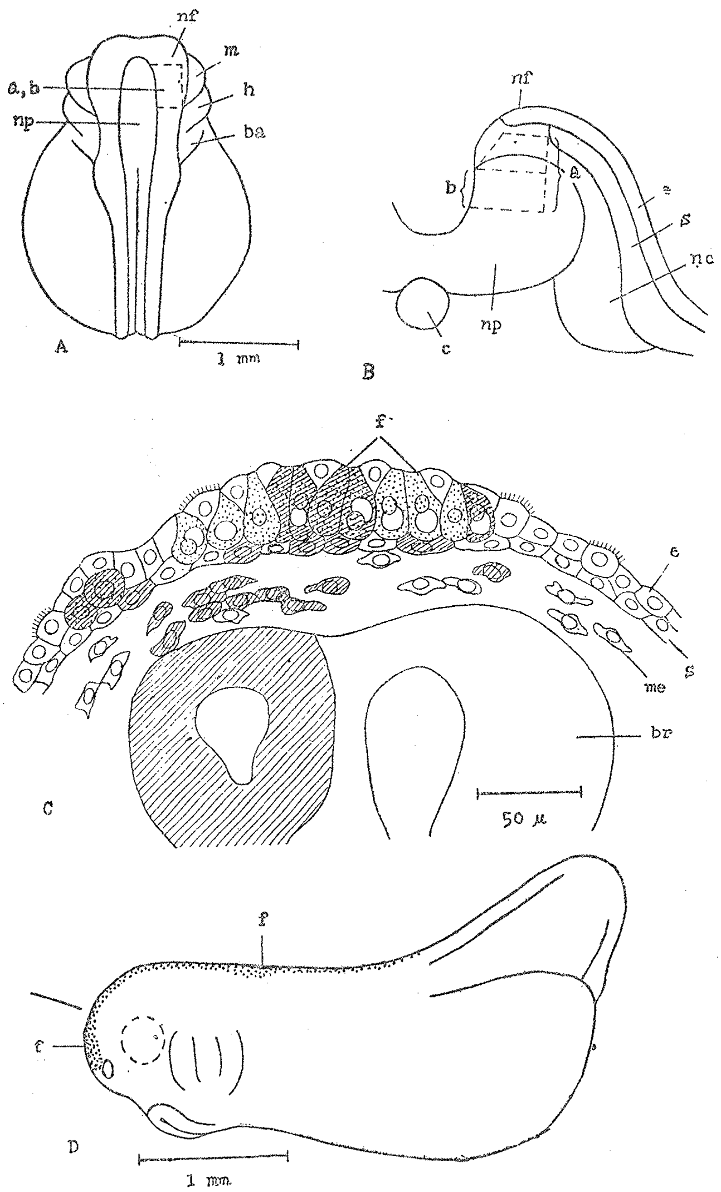

Fig. 2. Transplantation of Nile blue-stained neural crest in frog embryos. A, External appearance of neurula immediately before closure of neural groove. B, Section of anterior dorsal part of the same. Broken lines in Figs. A and B show planes of cutting at removal of transplant. a, Transplant composed of neural plate and neural crest. b, Transplant composed of neural plate only. C, Section of frontal region of embryo immediately before hatching, receiving Nile blue-stained transplant of neural plate plus neural crest. Frontal glands are dotted, vitally stained cells are shadowed. c, chorda; me, mesenchyme; np, neural plate. For other legends see Fig. 1.

Table 3.

Results of transplantation of Nile blue-stained neural plate only or together with neural crest in frog embryos

Case no.	Nile blue-stained transplant	No. of Nile blue-stained cells appearing outside of brain		
		Frontal gland	Basal layer of frontal gland	Mesenchyme
1	Neural plate plus neural crest	6	1	10
2	〃	23	28	24
3	〃	2	0	1
5	〃	3	0	7
11	Neural plate	0	0	0
12	〃	0	0	0
14	〃	0	0	0
15	〃	0	0	1
16	〃	0	0	0

exceptional case, No. 15, showed one Nile blue-stained cell among the mesenchyme. It is highly probable that, in this case, a few, perhaps one, neural crest cells were transplanted together with the neural plate piece.

Discussion and Conclusions

1. Formation of the frontal gland

As mentioned above, Holtfreter (1933) reported that frontal glands were formed in pieces of presumptive epidermis transplanted to the cephalic region of the neurula. He is of the opinion that the brain is primarily induced in the transplant by the neural crest of the host, and then the frontal glands became differentiated in the epidermis of the transplant under the influence of the inductive stimuli of the induced brain.

Hama (1946) succeeded in inducing frontal glands in the abdominal epidermis of the newt by means of fresh head-organizer or heat-killed trunk-organizer. Neither Holtfreter nor Hama, however, mentioned the role of the neural crest cells as the mother cells of the frontal glands.

On the other hand, Raven and Kloos (1945) have recently reported that the archenteron-roof is capable of inducing not only the neural plate but also the neural crest. According to these investigators, the neural crest is induced primarily by means of the lateral portion of the

archenteron-roof, while both the neural plate and neural crest are induced by the medial portion of the roof. It seems likely that, in the cases of Holtfreter and Hama, the primarily induced neural crest cells gave rise to the frontal glands after their migration into the epidermis.

After closure of the neural groove, the neural crest is found closely attached to the roof of the neural tube under the over-lying epidermis. It has been emphasized by McKeehan (1951) that the intimate contact of the organizer with the reacting tissue is usually a necessary condition of the occurrence of induction. The possibility that the neural crest cells are made to migrate to the epidermis and differentiate into frontal glands by an inductive stimulus originating from the brain remains to be settled.

2. Interpretation of experimental results

The frontal glands in the head region were reduced in number in toad embryos which had been deprived of the neural crest in the cephalic region in the earlier stage. In sham-operated embryos the glands were not decreased in number. It seems highly probable that, in the former, the neural crest cells which had thrust into the epidermis by the time of operation gave rise to the frontal glands, but the lack of a further supply of neural crest cells resulted in a reduction in number of the frontal glands. Mitoses are not found in the frontal glands, so that any increase in number of the glands must be regarded as the result of transformation of cells of another type into gland cells.

In the experiment with frog embryos, the Nile blue-stained frontal glands were found only in embryos which had received the vitally stained neural crest transplants. Stained cells also appeared in the basal layer of the frontal glands and in the mesenchyme of the host. If transplants composed of neural plate only had been given, no Nile blue-stained frontal glands appeared in the epidermis of the host. The results strongly support Yanai's previous conclusion that the frontal glands are of neural crest origin.

Indeed, the possibility that at least some of the frontal glands are formed from the epidermal cells has not yet been excluded. The observations of normal development of the frontal glands in various amphibians as well as the results of experimental analysis of their origin presented in this paper, however, have given no evidence of the epidermal origin of the glands.

SUMMARY

1. Experiments were performed with embryos at the stage of neural groove closure of the toad, *Bufo vulgaris formosus*, and the frog, *Rana*

nigromaculata nigromaculata, in order to ascertain the origin of the frontal glands.

2. In toad embryos which had been deprived of the cephalic neural crest, the frontal glands in the head region became reduced in number when compared with intact controls.

3. In frog embryos receiving a Nile blue-stained piece of the neural plate plus the neural crest, the stained cells appeared not only in the brain but also in the frontal glands, the basal layer of the glands, and the mesenchyme of the host's head. If a graft containing exclusively neural plate tissue had been given, vitally stained cells appeared only in the brain and not in other places of the host's head.

4. The results strongly support Yanai's previous conclusion that the frontal glands are of neural crest origin.

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